**ARIC Manuscript Proposal #3885**

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| **PC Reviewed: 7/13/21** | **Status: _____** | **Priority: 2** |
| **SC Reviewed: _____** | **Status: _____** | **Priority: ____** |

**1.a. Full Title**: Biological mediators and psychosocial moderators of the effects of neighborhood stressors on cardiovascular disease

**b. Abbreviated Title (Length 26 characters)**: Characterizing pathways from neighborhoods stressors to CVD

**2. Writing Group**:

Writing group members: ***Sharrelle Barber (JHS), Priya Palta, Shelly-Ann Love, James Pike, Anthony Zannas, Eric Boerwinkle, Gerardo Heiss (primary mentor, ARIC investigator)***

I, the first author, confirm that all the coauthors have given their approval for this manuscript proposal. __GB_ **[please confirm with your initials electronically or in writing]**


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**3. Timeline**: 2 years

**4. Rationale**:

**Social inequity-related chronic stress as a cause of cardiovascular disease disparities.** Although life expectancy and overall health have improved in the United States in recent years, these gains are not equally distributed across populations of persons of different racial, ethnic, gender, and socioeconomic backgrounds [1]. The contrast in cardiovascular disease (CVD) morbidity and mortality between Black and White persons is increasingly attributed to the distinct social conditions in which these groups age [1-4]; experiences of social adversity stemming from racial inequity are shown to have life-long consequences on health [1,2]. Racial

disparities in CVD are particularly evident in heart failure (HF), which occurs among Black men at notably younger ages than other populations [3,5]. Consequently, as continued CVD disparities remain a central public health concern [5], the National Institute on Aging has emphasized a need for research addressing the multilevel processes that yield unequal health outcomes over the life course between demographically diverse populations [6].

Previous work has identified gender differences in the effects of social exposures such as socioeconomic deprivation, residential segregation, and the built environment on CVD [2,4,5]. Yet, even as public health interventions have begun to address these environmental risk factors through, for example, neighborhood modifications, the mechanisms by which social environments act to increase risk for CVD are not fully clear. Furthermore, long-term investments in environmental modifications are often politically and economically challenging. Identifying individual-level factors that mediate or moderate the relationship between neighborhoods and CVD outcomes will both provide clarity on these mechanisms and may offer useful, additional public health intervention targets for mitigating racial disparities in cardiovascular health.

**Psychosocial factors as potential moderators.** Studies examining the relationship of subjective psychosocial factors such as perceived social cohesion [7] and social status-based stressors [8] as well as dispositional traits like affect [9] and optimism [10] with CVD have indicated potential differences in CVD risk across levels of these characteristics for women and men. These findings are consistent with theory outlining gender-related psychosocial influences on stress appraisal and health behaviors [11]. Inconsistencies within this literature, however, demonstrate that despite accurately identifying the structural contributions to inequitable CVD outcomes across racial groups, the specific ways in which external exposures are differentially internalized by women and men to shape physiological outcomes remain obscured. This lingering uncertainty challenges the efficacy of public health interventions and points to the need for additional studies examining how psychosocial characteristics shape the way individuals' cardiovascular health is impacted by the neighborhoods in which they grow. It is particularly important to examine factors that are theorized as protective (e.g. optimism) as well as those theorized as detrimental (e.g. negative affect) in seeking methods for promoting cardiovascular health and reducing disparities as these traits likely operate along different pathways [12].

**Epigenetic aging as a potential mediator.** The emerging field of geroscience offers additional avenues for understanding how the social environment is internalized to produce health, and, consequently, for investigating demographic divergence in the onset and severity of CVD. Geroscientific theory reconceptualizes chronic disease as a product of sustained dysfunctional aging [13]. Syndromes falling under the umbrella of CVD, many cancers, and accelerated neurodegeneration, for example, are seen not as distinct pathologies but as distinctly manifest age-related phenotypes sharing a core set of biological mechanisms which act over the life course [13,14]. Promising work emerging from this literature has identified several dimensions of aging susceptible to environmental stressors [15]. Of these, epigenetic mechanisms such as DNA methylation appear particularly responsive to psychosocial stressors [16]. A number of epigenetic "clocks", or composite measures of DNA modifications that predict chronologic age, health, and mortality, have been previously derived and validated [16]. As epigenetic alterations are only one modality through which to examine the multisystem aging process, there are other

important factors linking neighborhoods, psychosocial stressors, and aging to CVD [13]. Still, epigenetic clocks may serve as useful markers which can provide important missing information on the nature of these relationships and potentially new opportunities for intervention. As far as we have been able to determine, no studies have examined whether neighborhood environments impact on individual-level CVD risk through accelerating biological aging and further, whether individual psychosocial factors influence this process.

**The current study.** This study will capitalize on the rich data from participants in both ARIC Jackson and JHS to shed light on whether local social environments such as neighborhoods influence aging and cardiovascular health through epigenetic mechanisms, how psychosocial factors influence these relationships, and whether these mechanisms are consistent for both women and men. The specific objective of the proposed study is to assess whether biological age mediates the relationship between neighborhood dimensions and individual-level incident CVD, and whether this mediation is moderated by theorized psychosocial risk and resilience factors including negative affect, perceived social status, perceived impact of discrimination, optimism, spirituality, and religiosity. In doing so, our study may elucidate pathways from neighborhood contexts to cardiovascular outcomes and identify additional potential points of intervening on the disproportionate burden of CVD in black communities.

## 5. Main Hypothesis/Study Questions:

H1: Neighborhood psychosocial context and socioeconomic context independently predict biological age such that the more resource-deprived, the lower the perceived social cohesion, and the higher the perceived disorder of the neighborhood in which an individual lives, the higher their biological age relative to chronologic age.

H2: Psychosocial risk factors increase the effects, while psychosocial resilience factors attenuate the effects, of neighborhood context on biological age.

H3: Biological age partially mediates the effect of each neighborhood dimension on incident CVD (CHD, HF, or stroke).

H4: The association of neighborhood psychosocial and socioeconomic dimensions with biological age will differ by gender, such that men are more vulnerable to the effects of psychosocial factors while women are more vulnerable to the effects of socioeconomic factors.

## 6. Design and analysis (study design, inclusion/exclusion, outcome and other variables of interest with specific reference to the time of their collection, summary of data analysis, and any anticipated methodologic limitations or challenges if present).

Study sample: ARIC-JHS shared cohort (n=1625)

Sources of data: Epigenetic, CVD outcomes, age (ARIC); neighborhood variables, individual-level psychosocial factors, health behaviors (JHS)

**Primary exposure**
We will separately examine the effects of two dimensions of neighborhood (measured at the JHS baseline visit, 2000 - 2004): psychosocial context and socioeconomic context.

2. For socioeconomic context, we will compare two measures: 1) racial residential segregation, calculated at the census tract-level based on JHS methods using racial composition data (continuous, range 0 to 100), and 2) a previously derived neighborhood disadvantage score specific to the Jackson area [4]. This measure combines the following factors: % individuals living below federal poverty threshold, % households receiving public assistance, % occupied housing units with no vehicle, % adults aged 25 years and older with less than a high-school education, % unemployed individuals aged 16 years and older in the civilian labor force % unoccupied housing units, % occupied housing units with more than 1 person per room (crowding), and % female-headed households. The range for this continuous standardized score is -5 - 10.

**Covariates**
Health behaviors including binge-drinking (yes/no), cigarette smoking (never, previous, current), and leisure time physical activity (number of minutes, continuous) (hypotheses 1 and 4); income (below federal poverty line, yes/no) and education (number of years, continuous) (hypotheses 1-4). Other established CVD risk factors (e.g. hypertension, diabetes) are considered intermediaries and therefore will not be analyzed as confounders.

**Mediators**
Biological age operationalized as DNAm Age [17, 18] which has been previously calculated in ARIC [19], will be examined in relation to chronologic age. We will additionally calculate and examine PhenoAge [15] and GrimAge [20]. DNA was extracted from whole-blood white cells using the Gentra Puregene Blood Kit (Qiagen). One microgram of DNA underwent bisulphite conversion using the deep-well EZ-96 DNA Methylation Kit (Zymo Research); conversion efficiency was determined by polymerase chain reaction amplification using the Universal Methylated Human DNA Standard (Zymo Research). Methylation status was measured using the Illumina Infinium HumanMethylation450 BeadChip array (Illumina, Inc, San Diego, CA). Degree of methylation was determined using Illumina GenomeStudio 2011.1, Methylation module 1.9.0 software. The methylation score for each CpG was represented as a β value calculated by dividing the fluorescence intensity of the methylated bead type by the sum of the intensities of the methylated and unmethylated bead types. Background subtraction was conducted with the GenomeStudio software using built-in negative control bead types on the array. An average normalization was applied to minimize scanner-to-scanner variation. Investigators used the online calculator by Horvath [18] to perform additional normalization and imputation for missing β values and to estimate each of the Horvath and Hannum et. al. [19] versions of epigenetic age. Because heterogeneity in the composition of blood leukocyte cell types can confound relationships between DNA methylation and disease outcomes, they also used the online calculator to obtain cell type abundance measures as estimated from methylation data [19].

**Moderators**

Individual-level psychosocial variables (measured at baseline) including negative affect, perceived social status, optimism, spirituality, and religiosity.

Negative affect will be measured using summary scores of cynicism, anger-in, anger-out, and depressive symptoms. Cynicism is measured using items 1–13 of the Cook-Medley Hostility Scale, where participants were asked to answer "true or false" on such items as "… It is safer to trust nobody." We will calculate a total score (range 0–13), where higher scores indicate higher distrust. Anger will be assessed using a validated scale that measures anger-in and -out (both 8 items). Participants were asked how often they reacted to such items as "I express my anger" that were rated from almost never (1) to almost always (4). Anger-in and -out scores range from 0 to 23 and 0 to 22 respectively, where higher scores indicate higher anger. Depressive symptoms were measured using the 20-item Centers for Epidemiologic Studies Depression scale, where participants were asked about their mood, responding to items ("I was bothered by things that … don't bother me") about how often they felt this way. Items rated from 0 ("rarely/none of the time") to 3 ("most/all of the time"). This scale ranges from 0 to 60 with higher scores reflecting greater depressive symptoms.

Perceived social standing in the community will be operationalized as the individual's self-reported position in the social hierarchy of two different reference groups: a) the entire United States (U.S.) and b) the community with which the individual identifies. The U.S. social standing was measured by showing the participants a picture of a 10 rung ladder and asking the single question: "Now, think of a ladder with 10 steps representing where people stand in the United States. At step 10 are the people who are the best off—those who have the most money, the most education and the most respected jobs. At step 1 are the people who are the worst off--who have the least money, least education, and the worst jobs or no job." They were then asked to indicate which rung they would place themselves on. The question used for the community social standing was "Think of this ladder with ten steps as representing where people stand in their communities. People define community in different ways. Please define it in whatever way is meaningful to you. At step 10 are people who have the highest standing in their community. At step 1 are people who have the lowest standing in their community." For each of these reference groups, the gender-specific distribution of the ladder scores from 0–10 will be standardized into *z* scores by subtracting the population mean and dividing by the population standard deviation (SD) and used as a continuous variable.

The Life Orientation Test-Revised (LOT-R) scale, a validated measure of optimism, was completed at year 1. The LOT-R is a 6-item scale with a range of 6 (least optimistic) to 24 (most optimistic). Participants responded to 3 positively-worded items (e.g., "I'm always optimistic about my future") and 3 negatively-worded items (e.g., "If something can go wrong for me, it will"). In the total optimism score, the three positively-worded items will be reversed coded so that a higher score indicates higher optimism. The composite score will then be classified into tertiles (low, medium, high) to assess for threshold effects and continuously in standard deviation (SD) units.

Dimensions of religiosity will include organized religious activity and private prayer. Organized religious activity will be defined as church attendance or involvement in other forms of organized religion such as watching services on TV or participating in Bible study groups. Participants indicated the frequency of these activities as not at all, less than once a year, a few times a year, a few times a month, at least once a week, or nearly every day. These responses

will be coded from 1 to 6, respectively, with higher scores indicating more frequent attendance. Private religious experience will be assessed as reported frequency of prayer or meditation outside of formal religious activity (rated as never, less than once a month, once a month, a few times a month, once a week, a few times a week, once a day, or more than once a day). This item will be coded from 1 to 8, respectively, with higher scores indicating more frequent private prayer. This variable will be included in models as continuous.

Spirituality will be measured using the Daily Spiritual Experiences Scale (DSES), which assesses daily spiritual experiences in six domains, including feeling God's presence, feeling God's love, and being spiritually touched by creation and has been shown to have good psychometric properties in the JHS. Participants were asked to rate the frequency of these experiences from "never" to "many times a day," which will be coded as 1 to 5, respectively, and summed. The DSES score ranges from 5 to 30 with higher scores indicating higher spirituality. This variable will be included in models as continuous.

**Primary outcome**
Individual-level incident CVD measured as binary (yes/no) to HF, CHD, or stroke.

**Statistical analysis**
We will use multilevel moderated mediation analyses [21] to assess the extent to which and whether the relationships between neighborhood, epigenetic aging, and CVD is moderated by psychosocial factors.

Multilevel structural equation models (MSEM) will be employed to assess the crude and fully adjusted association between each neighborhood context and the ratio of DNAm Age (Horvath's and Hannum's), GrimAge, and PhenoAge to chronological age (hypothesis 1). Model evaluation and parameter estimation will be executed in Mplus utilizing full-information maximum likelihood (FIML) procedures with standard errors robust to non-normality. Multiple imputation by chained equations (MICE) with auxiliary variables will be performed for any missing data.

To test mediation of the hierarchical relationship between each neighborhood dimension and individual-level incident CVD by the ratio of DNAm Age, GrimAge, and PhenoAge to chronological age, as well as moderation of this mediation by psychosocial factors (hypotheses 2 and 3), multilevel moderated mediation models will be tested in Mplus using the same process outlined for the prior hypotheses. Indirect effects will be quantified with bias-corrected bootstrapping and 95% confidence intervals. All analyses will be stratified by gender (hypothesis 4). An exploratory analysis will evaluate whether a multiple group (female and male) MSEM can be fit to the data. ANOVA and Tukey's test for multiple comparisons will be used to address chance significance.

| Variables | Dataset | Level | Time point |
|---|---|---|---|
| CVD outcomes | ARIC | Individual | Follow-up through 2019 |
| Epigenetic data | ARIC | Individual | Ongoing, visits 2 and 3 (1990-1995) available so far |
| Health behaviors | JHS | Individual | Baseline (2000 – 2004) |

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|---|---|---|---|
| Neighborhood variables | JHS | Census-tract | Baseline |
| Psychosocial variables | JHS | Individual | Baseline |

**7.a. Will the data be used for non-ARIC analysis or by a for-profit organization in this manuscript? ____ Yes __X__ No**

**b. If Yes, is the author aware that the current derived consent file ICTDER05 must be used to exclude persons with a value RES_OTH and/or RES_DNA = "ARIC only" and/or "Not for Profit" ? ____ Yes ____ No**
(The file ICTDER has been distributed to ARIC PIs, and contains the responses to consent updates related to stored sample use for research.)

**8.a. Will the DNA data be used in this manuscript? __X__ Yes ____ No**

**8.b. If yes, is the author aware that either DNA data distributed by the Coordinating Center must be used, or the current derived consent file ICTDER05 must be used to exclude those with value RES_DNA = "No use/storage DNA"? __X__ Yes ____ No**

**9. The lead author of this manuscript proposal has reviewed the list of existing ARIC Study manuscript proposals and has found no overlap between this proposal and previously approved manuscript proposals either published or still in active status.**
ARIC Investigators have access to the publications lists under the Study Members Area of the web site at: http://www.cscc.unc.edu/aric/mantrack/maintain/search/dtSearch.html

___X___ Yes _____ No

**10. What are the most related manuscript proposals in ARIC (authors are encouraged to contact lead authors of these proposals for comments on the new proposal or collaboration)?**

The authors are not aware of overlapping or similar manuscript proposals.

**11.a. Is this manuscript proposal associated with any ARIC ancillary studies or use any ancillary study data? __X__ Yes ____ No**

**11.b. If yes, is the proposal**
**_X__ A. primarily the result of an ancillary study (list number* ___2020.23_____)**
**___ B. primarily based on ARIC data with ancillary data playing a minor role (usually control variables; list number(s)* _____ _____ _____)**

*ancillary studies are listed by number https://sites.cscc.unc.edu/aric/approved-ancillary-studies

**12a. Manuscript preparation is expected to be completed in one to three years. If a manuscript is not submitted for ARIC review at the end of the 3-years from the date of the approval, the manuscript proposal will expire.**

**12b. The NIH instituted a Public Access Policy in April, 2008** which ensures that the public has access to the published results of NIH funded research. It is **your responsibility to upload manuscripts to PubMed Central** whenever the journal does not and be in compliance with this policy. Four files about the public access policy from http://publicaccess.nih.gov/ are posted in http://www.cscc.unc.edu/aric/index.php, under Publications, Policies & Forms. http://publicaccess.nih.gov/submit_process_journals.htm shows you which journals automatically upload articles to PubMed central.


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